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# 睡莲科的系统发育:核糖体 DNA ITS 区序列证据

刘艳玲　徐立铭　倪学明* 赵家荣
(中国科学院武汉植物园　武汉 430074)

# Phylogeny of the Nymphaeaceae inferred from ITS sequences

LIU Yan-Ling　XU Li-Ming　NI Xue-Ming*　ZHAO Jia-Rong
(*Wuhan Botanical Garden, the Chinese Academy of Sciences*, Wuhan 430074, China)

**Abstract** The Nymphaeaceae are one of the most important taxa for understanding the origin and evolution of primitive angiosperms due to its basal position in the cladograms of the angiosperms recently given based both on several gene sequences and on morphological characters, but phylogenetic relationships within the family have not as yet been resolved. The sequences of nrDNA ITS region of 11 species representing seven genera of the Nymphaeaceae and one outgroup, *Ceratophyllum demersum*, were used to reconstruct the phylogeny of the family using PAUP4.0b4A. Three most parsimonious trees (Length=1125, *CI*=0.7618 and *RI*=0.7214) were obtained. In the consensus tree: (1) *Nelumbo* was basal to the other genera with a bootstrap value of 100% and occupied an isolated position, so it could be separated from the Nymphaeaceae and placed in its own family, Nelumbonaceae, and its own order, Nelumbonales; (2) *Nuphar* was monophyletic, nested in the basal position of clade II and was strongly supported to be the sister group of the other genera (excluding *Nelumbo*) of the Nymphaeaceae, suggesting that its traditional inclusion in the Nymphaeaceae should be maintained; (3) *Cabomba* and *Brasenia* were sister group and formed a subclade with a bootstrap value of 99%, indicating their close affinity; (4) *Nymphaea* was strongly supported to be the sister group of a subclade comprising *Euryale* and *Victoria* with a bootstrap value of 94%, indicating that they should be placed in the Nymphaeaceae.

**Key words** Nymphaeaceae, ITS, phylogeny.

摘要　以金鱼藻*Ceratophyllum demersum*为外类群,使用PAUP4.0b4A软件对睡莲科Nymphaeaceae植物7属11个代表种的ITS区序列进行了系统发育分析。采用最大简约法分析获得了3个最简约树,步长为1125,一致性指数(*CI*)和维持性指数(*RI*)值分别为0.7618和0.7214。利用3个最简约树获取严格一致树。结果表明:(1)莲属*Nelumbo*位于系统树的基部,自展支持率为100%,可从睡莲科中独立出来成立莲科Nelumbonaceae和莲目Nelumbonales;(2)萍蓬草属*Nuphar*是一单系类群,位于分支Ⅱ的基部,并和睡莲科其他属(不包括莲属)植物聚在一起构成姐妹群,故萍蓬草属仍应置于睡莲科中;(3)水盾草属*Cabomba*和莼菜属*Brasenia*聚成一小支并构成姐妹群,自展支持率为99%,说明这两属之间亲缘关系较近;(4)睡莲属和芡实属*Euryale*、王莲属*Victoria*聚成一小支并构成姐妹群,自展支持率为94%,说明三者亲缘关系较近,仍应置于睡莲科中。

关键词　睡莲科; ITS; 系统发育

睡莲科Nymphaeaceae有9属约100种,广布于温带与热带地区(唐先华等, 2003)。随着新归化属植物——水盾草*Cabomba caroliniana* A. Gray在浙江杭州、宁波、鄞县等地的发现

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* 通讯作者(Author for correspondence. E-mail: aquaticplant@wbgcas.cn)。

(丁炳扬, 2000),睡莲科在中国的分布或栽培已有7属:睡莲属*Nymphaea* L.、莲属*Nelumbo* Adans.、萍蓬草属*Nuphar* J. E. Smith、芡实属*Euryale* Salisb. ex DC.、王莲属*Victoria* Lindl.、水盾草属*Cabomba* Aubl.、莼菜属*Brasenia* Schreb.。另外不见于我国的两属为*Ondinea* Hartog (Hartog, 1970; Helen, 1977)和*Barclaya* Wall. (Bentham & Hooker, 1862)。

形态和分子证据表明睡莲科是被子植物谱系的最早分支之一(Barkman et al., 2000; Soltis et al., 2000),也是原始双子叶植物中的一个重要类群,隶属于毛茛目,又兼有单子叶植物的若干性状,对探讨被子植物系统演化及单、双子叶植物起源等问题具有重大意义,因而倍受关注。国内外学者已从形态学(Osborn & Schneider, 1988; Moseley et al., 1993; 倪学明等, 1994; Les et al., 1997)、解剖学(陈维培,张四美, 1992; Moseley et al., 1993; Schneider et al., 1995; Schneider & Carlquist, 1996a, b; Hayes et al., 2000; 胡光万等, 2003)、孢粉学(Walker, 1974, 1976; 张玉龙, 1984; Osborn et al., 1991)、染色体数及核型(韦平和等, 1993, 1994)、酯酶同功酶(陆佩洪,陈维培, 1993; 陆佩洪等, 1994)等方面做了大量的工作,但并未取得一致意见。有的学者主张作为一科处理,即睡莲科(Bentham & Hooker, 1862; Caspary, 1891);或两科处理,即莲科Nelumbonaceae或Cabombaceae和睡莲科(Hutchinson, 1959; Les, 1991);或三科处理,即Cabombaceae、睡莲科和莲科(Takhtajan, 1980; Cronquist, 1988; Williamson, 1993a, b; Les, 1999); 还有的主张增加一萍蓬草科Nupharaceae(Takhtajan, 1997)作为四科处理等不同观点。在基于大量分子资料而建立的APG(The Angiosperm Phylogeny Group)系统(汤彦承等, 1999)中,把莲科置于山龙眼目Proteales下,取消了睡莲目Nymphaeales,并把Cabombaceae合并到睡莲科中。而吴征镒等(2003)则支持将莲科升为莲目Nelumbonales隶属于毛茛纲,而睡莲目仍置于木兰纲,并调整为4个科:莼菜科Hydropeltidaceae,竹节水松科Cabombaceae、睡莲科和芡科Euryalaceae。有关睡莲科科内的分子系统发育及属间谱系分支研究较少。Les等(1991)以单基因*rbc*L序列构建了睡莲科植物的分子系统发育树,支持将莲属独立出来成立莲科,隶属于莲目。利用*rbc*L、*mat*K和18S rDNA序列,并结合重要的形态学性状重建的睡莲科植物(不包括莲属)系统发育树,又支持将水盾草属和莼菜属独立出来成立Cabombaceae (Les et al., 1999),仍置于睡莲目。唐先华等(2003)仅获得了睡莲科4属植物的ITS序列,初步探讨了ITS序列在睡莲科植物系统发育分析中的应用。

核糖体DNA中的内转录间隔区(ITS)序列由于可以提供较丰富的信息位点,已被广泛用于研究被子植物属内种间、近缘属间乃至科内的系统发育和分类问题(Baldwin et al., 1995; 刘忠等, 2000; 田欣,李德铢, 2002)。本文通过对睡莲科7属11种植物及外类群金鱼藻*Ceratophyllum demersum* L.的nrDNA ITS序列分析,构建了睡莲科植物的ITS系统发育树,并在此基础上结合一些重要的形态性状,探讨了科内的谱系发生关系,同时也试图为睡莲科植物的分类学位置研究提供新的证据。

## 1 材料和方法

### 1.1 材料

实验材料包括睡莲科植物7属11种及外类群金鱼藻,其来源及基因库样品序号见表1。

表1 材料来源
**Table 1** Origin of materials

| 分类群<br>Taxon | 产地<br>Provenance* | 凭证标本<br>Voucher | GenBank 检索号<br>GenBank Acc. No. |
|---|---|---|---|
| 中国莲<br>*Nelumbo nucifera* Gaertn. Fruct. & Semin. | 中国科学院武汉植物园(栽培)<br>WBGCAS (cultivated) | 刘艳玲 (Y. L. Liu) 20042 | AY620418 |
| 美洲黄莲<br>*Nelumbo lutea* (Willd.) Pers. | 中国科学院武汉植物园(栽培)<br>WBGCAS (cultivated) | 刘艳玲 (Y. L. Liu) 20043 | AY620419 |
| 埃及蓝睡莲<br>*Nymphaea coerulea Sav.* | 中国科学院武汉植物园(栽培)<br>WBGCAS (cultivated) | 刘艳玲 (Y. L. Liu) 20044 | AY620420 |
| 南非蓝睡莲<br>*Nymphaea capensis* Thunb. | 中国科学院武汉植物园(栽培)<br>WBGCAS (cultivated) | 刘艳玲 (Y. L. Liu) 20045 | AY620421 |
| 芡实<br>*Euryale ferox* Salisb. ex Konig & Sims | 中国科学院武汉植物园(栽培)<br>WBGCAS (cultivated) | 刘艳玲 (Y. L. Liu) 20048 | AY620422 |
| 克鲁兹王莲<br>*Victoria cruziana* Orb. | 中国科学院武汉植物园(栽培)<br>WBGCAS (cultivated) | 刘艳玲 (Y. L. Liu) 20049 | AY620423 |
| 水盾草<br>*Cabomba caroliniana* A. Gray | 浙江杭州<br>Hangzhou, Zhejiang | 赵家荣 (J. R. Zhao) 20037 | AY620424 |
| 红菊花草<br>*Cabomba piauhyensis* Gardn. | 武汉港花鸟市场<br>Bought from the Flower-and-Bird Market of Wuhan Port | 徐立铭 (L. M. Xu) 20031 | AY620425 |
| 莼菜<br>*Brasenia schreberi* J. F. Gmel. | 中国科学院武汉植物园(栽培)<br>WBGCAS (cultivated) | 刘艳玲 (Y. L. Liu) 20040 | AY620426 |
| 中华萍蓬草<br>*Nuphar sinensis* Hand.-Mazz. | 中国科学院武汉植物园(栽培)<br>WBGCAS (cultivated) | 刘艳玲 (Y. L. Liu) 20047 | AY620428 |
| 贵州萍蓬草<br>*Nuphar bornetii* Lévl. & Vant. | 中国科学院武汉植物园(栽培)<br>WBGCAS (cultivated) | 刘艳玲 (Y. L. Liu) 20046 | AY620427 |
| 金鱼藻<br>*Ceratophyllum demersum* L. | 中国科学院武汉植物园(栽培)<br>WBGCAS (cultivated) | 刘艳玲 (Y. L. Liu) 20041 | AY620429 |

* WBGCAS, Wuhan Botanical Garden, the Chinese Academy of Sciences.

### 1.2 总 DNA 提取、PCR 扩增和 DNA 测序

取上述植物的新鲜叶片约0.1 g,采用改良的CTAB法(Doyle & Doyle, 1987)提取总DNA。聚合酶链式反应(PCR)在MJ“PTC-225”型PCR仪上进行,用White等(1990)描述的一对通用引物ITS4和ITS5进行ITS区的扩增(ITS4序列:5'TCCTCCGCTTATTGATATGC 3',ITS5序列:5'GGAAGTAAAAGTCGTAACAAGG3')。PCR反应程序为:94 ℃预变性4 min,94 ℃变性1 min,50 ℃退火1 min,72 ℃延伸2 min,30个循环;扩增产物经回收纯化后克隆到pMD 18-T载体上并在含有X-Gal、IPTG和Amp的L-琼脂平板培养基上培养,挑选出阳性克隆送上海生工生物工程技术有限公司用ABI377型自动测序仪进行序列测定,测序引物为M13+/M13-。为了保证测序的准确性,对ITS序列正、反链进行测定并加以校准。

### 1.3 序列分析和系统树的构建

本文测定的序列经Clustal X (Thompson et al., 1997)软件排序,个别位点做了必要的人工校正,以金鱼藻为外类群,用PAUP 4.0b4A(Swofford, 1998)软件进行系统发育分析。空位(gap)被编码为缺失(missing)。最大简约性分析采用如下选项完成,即:树二组重新连接

(TBR)、启发式搜索(Heuristic search)、多重性选择(MULTREES option)、ACCTRAN优化和100次随机附加的重复。利用自展分析(bootstrap, 1000次重复)(Felsenstein,1985)检验各分支的置信度。

## 2　结果和分析

如表2所示:本文测得的11种睡莲科植物的ITS1序列长度范围为257-274 bp,GC百分含量为40.83%-63.12%,ITS2为244-274 bp,GC百分含量为44.12%-63.26%,5.8S rDNA为156 bp(水盾草插入两个碱基,5.8S rDNA为158 bp),整个ITS区的长度范围为660-704 bp。当空位作缺失处理时,ITS区全序列排序后的长度为730位点,其中有114个变异位点,68个为系统发育的信息位点,分别占15.62%和9.32%。简约性分析得到3个最简约树(MPTs),其步长为1125,一致性指数(*CI*)和维持性指数(*RI*)分别为0.7618和0.7214。

**表2**　11种睡莲科植物的ITS长度和G+C含量

**Table 2**　The size and G+C content of ITS of the 11 species of the Nymphaeaceae studied

| 分类群 | ITS | ITS1 | | ITS2 | |
|---|---|---|---|---|---|
| Taxon | Size (bp) | Size (bp) | G+C (%) | Size (bp) | G+C (%) |
| 中国莲 *Nelumbo nucifera* | 673 | 260 | 54.62 | 257 | 57.98 |
| 美洲黄莲 *Nelumbo lutea* | 675 | 257 | 56.03 | 262 | 59.16 |
| 埃及蓝睡莲 *Nymphaea coerulea* | 701 | 274 | 47.45 | 271 | 51.09 |
| 南非蓝睡莲 *Nymphaea capensis* | 704 | 274 | 45.62 | 274 | 50.36 |
| 芡实 *Euryale ferox* | 660 | 259 | 40.83 | 244 | 44.12 |
| 克鲁兹王莲 *Victoria cruziana* | 678 | 258 | 42.25 | 264 | 50.00 |
| 水盾草 *Cabomba caroliniana* | 698 | 269 | 58.74 | 271 | 56.09 |
| 红菊花草 *Cabomba piauhyensis* | 697 | 262 | 58.02 | 279 | 58.42 |
| 莼菜 *Brasenia schreberi* | 683 | 263 | 63.12 | 264 | 63.26 |
| 贵州萍蓬草 *Nuphar bornetii* | 676 | 257 | 55.64 | 263 | 58.56 |
| 中华萍蓬草 *Nuphar sinensis* | 665 | 259 | 54.83 | 250 | 55.20 |

利用3个最简约树获取的严格一致树如图1所示。睡莲科7属11个物种分别聚成两支,且分别获得100%的自展支持率。分支I为莲属的2个种,位于系统发育树的基部(自展支持率为100%)。睡莲科的其他6属聚成一支,即分支II,这一支又包括2个亚支,其中萍蓬草属的2个代表种以100%的自展支持率聚在一起,并位于相对基部的位置;另外一个亚支又分成2个小支。其中睡莲属、王莲属、芡实属和莼菜属、水盾草属分别聚成一小支并构成姐妹群,且获得了较高的自展支持率(94%和99%),但这两小支聚在一起的支持强度却不高(61%)。

## 3　讨论

### 3.1　莲属的分类等级及其系统位置

莲属由于具有离生心皮、无导管木质部、双层珠被、厚珠心的倒生胚珠、八核蓼型胚囊、叶片状雄蕊、层状胎座等原始特征而被置于睡莲科(韦平和等, 1994)。近年来,许多学者根据莲属独有的植物化学、形态学和细胞学特征(Takhtajan, 1969; 韦平和等, 1994; 倪学明

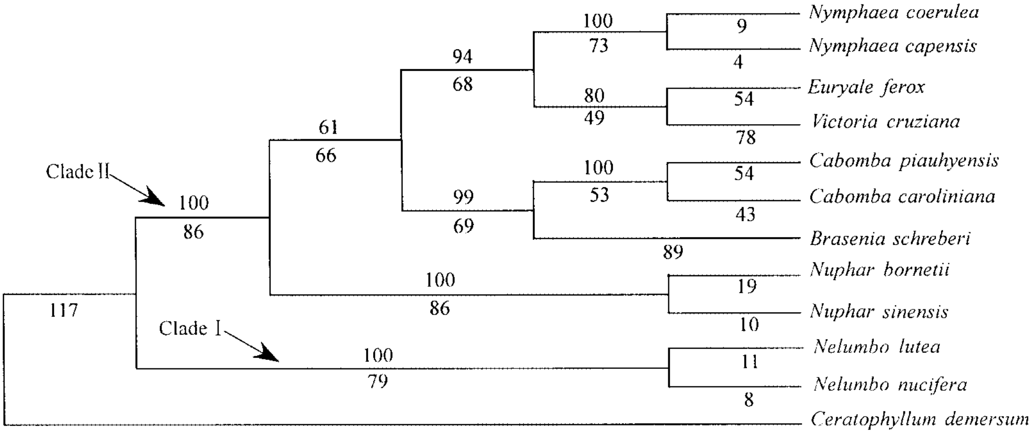

图**1** 基于ITS序列分析得到的3个最简约树的严格一致树(步长为1125,一致性指数(*CI*)0.7618,维持性指数(*RI*)为0.7214)。分支下面的数值代表支长变化,分支上面的数值代表自展支持率。

**Fig. 1.** The strict consensus tree of three equally most parsimonious trees based on ITS sequences with all characters equally weighted (Length=1125, *CI*=0.7618, *RI*=0.7214). Numbers below branches are base substitutions; those above branches are bootstrap values (%) with 1000 replicates.

等, 1994),支持莲属从睡莲科中独立出来成立莲科和莲目:莲属含苄基异喹啉类生物碱,果实具呼吸孔,种子单粒、无胚乳、两个子叶基部愈合,莲属叶片具排水器和厚壁异细胞、上下两表皮有气孔,属单唇型发育(Gupta et al., 1968);莲属花粉为三沟花粉、外壁具明显的柱状层结构;睡莲科中的分类群是由古多倍体起源的,而只有莲属的染色体基数x=8,可推断为古二倍体(韦平和等, 1993),同时它的染色体形态也显著不同于睡莲科其他类群,集中表现在莲属具有一对大的近端着丝点染色体、第2号染色体的一条长臂上存在明显的次缢痕以及可能出现的B染色体。

基于*rbc*L基因(Les et al., 1991)和本文ITS序列的分析表明:莲属是一单系类群,位于分支图的最基部(自展支持率为100%),应为睡莲科的原始类群(图1)。鉴于莲属独有的形态、细胞学特征及来自*rbc*L、ITS序列的分子证据,作者支持将它从睡莲科中独立出来成立莲科,隶属于莲目(Les et al., 1991; Li, 1955)。

### **3.2** 萍蓬草属的分类等级及其系统位置

萍蓬草属广布北半球的温带地区。形态解剖学的观察结果(Ito, 1987; Moseley et al., 1993; Philbrick & Les, 1996)和基于*rbc*L基因的序列分析(Les et al., 1991)都支持萍蓬草属的传统分类地位,即隶属于睡莲科(Caspary, 1891; Cronquist, 1988)。但最近的研究表明:萍蓬草属具有一些与睡莲科其他类群明显不同的特征(施国新等, 1998; 胡光万等, 2003; Moseley, 1965; Padgett et al., 1999),有分离出来独立成科的可能性。这些特征主要包括:(1)该属是睡莲科中惟一没有热带种类的一个属;(2)果实不沉入水中成熟;(3)子房上位;(4)远轴花瓣上分布有蜜腺;(5)花粉无沟或槽,饰孔多刺;(6)花梗中仅具一个星状排列的维管束;(7)叶柄基本组织中无气道;(8)根顶端原始细胞具有分层特征,属封闭型。陆佩洪等(1994)通过对睡莲科叶片中酯酶同功酶的测定也发现:萍蓬草属具有3-4条独有的酶谱,显示出与其他属不同的特征。

萍蓬草属与睡莲科其他类群之间有着明显的差异,但它们在具根状茎,叶二型,花两性、辐射对称、单生花梗顶端,萼片、花瓣均为3至多数,雄蕊6至多数、纵裂,倒生胚珠1至多数等方面是基本一致的(倪学明等, 1994)。Les等(1999)基于*rbc*L、*mat*K和18S rDNA的基因序列分析,并结合一些重要的形态性状,探讨了睡莲目植物的系统发生关系。其中萍蓬草属隶属于睡莲科(不包括莲属、莼菜属和水盾草属),位于睡莲科植物系统发育树的基部,并和*Barclaya*属保持较近的亲缘关系。本文的ITS序列分析结果也显示:萍蓬草属是一单系类群,位于分支II(图1,Clade II)的基部,并和睡莲科其他属(不包括莲属)植物以100%的自展支持率聚在一起构成姐妹群,故我们认为萍蓬草属应置于睡莲科中,保持其传统的分类地位(Caspary, 1891; Cronquist, 1988)。

### **3.3**　水盾草属和莼菜属的分类等级及其系统位置

水盾草属主要分布在新大陆的热带和温带地区,莼菜属集中分布在北纬40 °以南的亚热带地区(于丹, 1991),但两者在形态、花部、营养器官的解剖特征及孢粉学等方面有一些睡莲科其他属植物不具有的共征(胡光万等, 2003; Ito, 1986; Osborn et al., 1991):(1)除具有根状茎外,还具有沉水茎;(2)二者均为背生胎座;(3)花托维管束由带状束顺着花瓣、雄蕊形成分支的花托维管束组成(倪学明等, 1994);(4)单沟花粉;(5)花丝短而背腹扁平;(6)叶柄中只有一个双韧维管束,位于叶柄的中央;(7)叶柄和叶肉中无星状石细胞和草酸钙结晶。基于这些共征,许多学者都赞同将水盾草属和莼菜属共同置于一新科——Cabombaceae中(Hutchinson, 1959; Osborn et al., 1991; Schneider & Carlquist, 1996; Les et al., 1999)。并根据其具下垂倒生、两层珠被、厚珠心的胚珠和维管束中有原生木质部空腔等事实,将Cabombaceae仍置于睡莲目(Takhtajan, 1987; Cronquist, 1988)。

ITS序列的分析结果表明(图1):水盾草属和莼菜属聚成一小支,并以99%的自展支持率构成姐妹群,说明这两属之间亲缘关系较近,聚在一起是十分自然的,这和来自*rbc*L、*mat*K和18S rDNA基因序列的分子证据(Les et al., 1999)是相吻合的。同时水盾草属、莼菜属又和王莲属、芡实属、睡莲属以61%的自展支持率聚在一起构成姐妹群,说明它们之间存在一定的亲缘关系,故我们支持将水盾草属、莼菜属置于睡莲科中。

### **3.4**　睡莲科其他属的分类等级及其系统位置

芡实属和王莲属植物叶片硕大,叶柄、花梗及果实有刺,两者不仅在外部形态上极为相似,而且在其他方面还有许多共同特征:二者都有雄蕊瓣化现象、柱头辐射排列、下位子房与花托结合在一起、环槽花粉等(Swindells, 1983; 倪学明等, 1994)。对过氧化物酶酶谱的类平均聚类分析也显示王莲属与芡实属有较近的亲缘关系(陆佩洪,陈维培, 1994)。来自*rbc*L、*mat*K、18SrDNA(Les et al., 1991, 1999)及本文的ITS序列分析都表明:两者亲缘关系较近,并以较高的自展支持率聚在一起构成姐妹群。

陈维培等(1992)通过对睡莲科植物叶片的比较解剖发现:睡莲属在气孔器、腺毛、吸水器、星状石细胞、草酸钙结晶及维管束结构和排列方式、染色体形态上和芡实属、王莲属更为接近(韦平和等, 1994)。ITS序列分析也表明:睡莲属和王莲属、芡实属聚成一小支,并得到了较高的自展支持率(94%)(图1)。基于形态、比较解剖学等方面的共征以及ITS序列的分子证据,我们认为芡实属、王莲属和睡莲属三者亲缘关系较近,且染色体核型均为2A类型(韦平和等, 1994),仍应置于睡莲科中。

睡莲科植物不同属之间虽存在着较大的差异,但在盾叶的形式和发育进程、维管束的结构和排列方式、只有管胞而无导管、双层珠被、子叶2片、上胚轴较短而下胚轴模糊等方面是基本一致的,这说明它们之间并非仅是由于水生环境而被归在一起的,其间确实存在一定的亲缘关系。

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